Disinfection of Apples, Fresh-Cut Melons and Vegetable Sprouts with Hydrogen Peroxide

Gerald M. Sapers, Robert L. Miller, Vlasta Pilizota, and Angela M. Mattrazzo
Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Wyndmoor, PA

ABSTRACT

Recent *Escherichia coli* O157:H7 outbreaks associated with unpasteurized apple juice have demonstrated a need for improved methods of washing and sanitizing apples. A 200 ppm Cl_2 (pH 6.5) wash and representative commercial wash formulations reduced the *E. coli* population of inoculated apples by only 99%. However, experimental washes containing 5% hydrogen peroxide (H_2O_2) in combination with commercial sanitizing agents, heated to 50°C, achieved a population reduction in excess of 4 logs (99.99%). Fresh-cut melons are subject to rapid spoilage and may be contaminated with human pathogens. Bacteria on external melon surfaces are resistant to treatment with hypochlorite and commercial sanitizers. Bacterial populations on cantaloupe rind could be reduced by 2-3 logs (99-99.9%) by treatment with combinations of 5% H_2O_2 and commercial washing formulations at 50-60°C. The microbiological quality of fresh-cut cantaloupe cubes could be improved by washing whole melons with a surfactant-based sanitizer, followed by a second wash in 5% H_2O_2 or 2000 ppm Cl_2 prior to cutting. Sprouted seeds have been associated with *Salmonella* and *E. coli* O157:H7 outbreaks world-wide. Efforts to decontaminate seeds with anti-microbial agents such as hypochlorite have been limited in success, and surviving bacterial contaminants can grow to large numbers (10^6-10^9 cfu/g) during sprout production. Fully grown sprouts cannot be decontaminated by treatment with H_2O_2. These results demonstrate the potential value of H_2O_2 in disinfecting fresh fruit and vegetable products. Further research is needed to optimize treatments, determine their cost, and obtain regulatory approval.

INTRODUCTION

Fresh and minimally processed fruits and vegetables have been associated with a number of documented food poisoning outbreaks (Anon., 1996). Unpasteurized apple juice has been the vehicle of several recent outbreaks of *E. coli* O157:H7 infection in the U.S. Minimally processed (fresh-cut) cantaloupe melon has been associated with outbreaks of Salmonellosis (Beuchat, 1996). Efforts to improve the shelf-life and microbiological quality of fresh-cut cantaloupe have focused on reduction of microbial populations on the melon rind by treatment with hypochlorite (Ayhan et al., 1996; Cantwell et al., 1996) or hydrogen peroxide (Simmons et al., 1996). Recent

Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

E. coli O157:H7 outbreaks in Japan, involving thousands of cases and some fatalities, are now believed to be due, at least in part, to infected radish sprouts, while a 1997 outbreak in the U.S. was associated with alfalfa sprouts (Anon., 1997). Sprouted vegetable seeds and alfalfa sprouts have been associated with outbreaks of *Salmonella* in the U.S. (Mahon, 1997) and other countries (O'Mahony et al., 1990; Pönkä et al, 1995). Conditions used to produce sprouts are favorable to growth of pathogenic and other bacteria (Splittstoesser et al, 1983; Prokopowich and Blank, 1990).

Chlorine is widely used to sanitize and disinfect fresh and minimally processed fruits and vegetables (Brackett, 1992). However, while chlorine treatment reduced microbial populations on cut produce, it did not completely inactivate *Salmonella montevideo* on whole tomatoes at recommended chlorine concentrations (Zhuang et al., 1995; Wei et al., 1995) or substantially reduce total bacterial counts on fresh-cut carrots, red cabbage or lettuce in a commercial produce plant (Garg et al., 1990). Chlorine's effectiveness is limited by its reaction with organic material and protection of microorganisms by biofilms on the commodity surface.

Studies carried out in our laboratory have indicated that treatment with hydrogen peroxide can reduce microbial populations and extend the shelf-life of washed, fresh mushrooms (Sapers et al., 1994) and several fresh-cut vegetables and melons (Sapers et al., 1995). The objective of our research has been to develop more effective means of reducing microbial populations in fresh and minimally processed products including unpasteurized apple juice, fresh cut cantaloupe and sprouts.

MATERIALS AND METHODS

Apple decontamination studies. For each treatment, sets of 9 cold, unwaxed Golden Delicious apples were cut in half along the core axis and inoculated by immersion for 5 min in 3L of a diluted *E. coli* (ATCC 25922, 11775 or 23716) or *Enterobacter aerogenes* B-199 inoculum containing approx. 10^7 cfu/mL. The inoculated apple halves were washed by immersion in 2 L of the washing medium on a shaker for 1 min at ambient temperature or 50-60°C. Washed apple halves or unwashed, inoculated controls were homogenized with 2 L H_2O, and the homogenates were diluted and surface plated on Brain Heart Infusion Agar (BHIA), incubated at 36°C for 24 h, for enumeration of *E. coli* remaining in the inoculated fruit. This protocol was used to compare a series of commercial and experimental hydrogen peroxide-based washing formulations for apples vs. 200 ppm Cl_2 (pH adjusted to 6.5 with citric acid). Residual H_2O_2 concentrations in treated apples and juice made therefrom were determined by the Reflectoquant analysis system (EM Science, Gibbstown, NJ).

Cantaloupe decontamination studies. Sets of 10 cantaloupe rind plugs, 20 mm in diameter, were immersed in 500 mL wash solution with stirring for 5 min. Treatments included washes in various commercial surfactant/sanitizer solutions and experimental combinations of 5% H_2O_2 with the commercial formulations. Treated plugs were drained, rinsed with sterile H_2O, and homogenized with 50 mL sterile H_2O in a Waring blender for microbiological evaluation on Pseudomonas Agar F (Difco). Bacterial counts were expressed as cfu/cm^2 of surface area, based on the total external surface of the 10 plugs (31.4 cm^2).

In experiments with fresh-cut cantaloupe, 2-3 whole melons were sanitized by immersion for 2 min in solutions of either 1000 or 2000 ppm Cl_2 or 5% H_2O_2 containing various commercial

surfactant/sanitizing agents, followed by a water rinse. The flesh from treated melons was cut into cubes, and ~200g portions were packed into plastic boxes and heat-sealed within laminated polyethylene film bags (Cypress Packaging, Rochester, NY) having a specified OTR value of 1395 cc/m^2/day to provide modified atmosphere packaging (MAP). Replicate samples were stored at 4°C and examined at intervals for visual indications of spoilage and off-odors. Samples were homogenized with 250 mL 0.1% peptone, diluted with 0.1% peptone, and plated on Pseudomonas Agar F to determine total aerobic plate counts.

Sprout decontamination studies. Sprout samples, obtained from a commercial grower, were treated and evaluated within 1 day. Approximately 150g of each sample were immersed in a 200 ppm or 5% solution of hydrogen peroxide for 1 min. The treated sprouts were rinsed with H_20 and drained. Subsamples weighing ~40g were blended with 250 mL H_20 at high speed for 1 min, diluted with H_20, and plated on KB for total aerobic plate count (APC). Additional 10g subsamples were blended with 50 mL H_20, and the homogenates were strained through glass wool and analyzed for ascorbic acid by the EM Reflectoquant procedure.

RESULTS AND DISCUSSION

***Decontamination of apples inoculated with* E. coli.** A series of commercial washing formulations (Table 1) was compared with 200 ppm Cl_2 (pH 6.5) in decontamination trials with apple halves inoculated with generic *E. coli* strain ATCC 25922 (~10^5 cfu/g). Three products showed promise: 1% Sanitizer D (an acidic formulation), 4% Sanitizer G (trisodium phosphate), and 1000 ppm Sanitizer I (a peracetic acid formulation), each heated to 50°C. These achieved a 2.5 log reduction in the *E. coli* load, compared to a 2 log reduction for 200 ppm Cl_2 and <1 log reduction with a water wash (data not shown). However, heated (50°C) 5% H_2O_2 or combinations of 5% H_2O_2 with 5% Sanitizer B, 1 or 2% Sanitizer D, 1.6% Sanitizer E, or 3.2% Sanitizer F approached or exceeded a 4 log population reduction (Table 2). While combinations of H_2O_2 with 2% Sanitizer G (trisodium phosphate) were effective in decontaminating apples, variable results were obtained at 50°C, probably because of the instability of alkaline H_2O_2 solutions. Therefore, these combinations would not be suitable for use as a sanitizing treatment. Apples inoculated with *E. aerogenes* and two additional generic *E. coli* strains (ATCC 11775 and 23716) showed some variation in response to the washing treatments. Log reductions varied between 1.4 and 2.0 for 200 ppm Cl_2, between 2.3 and 3.3 for 5% H_2O_2 at 50°C, and between 2.6 and 4.1 for 5% H_2O_2 + 2% Sanitizer D at 50°C. Further study is required to determine whether such variation is due to differences in the acid tolerance of the bacterial strains.

Homogenized or cut, untreated apples normally contain low concentrations of endogenous H_2O_2 as a result of enzymatic reactions in disrupted apple tissue. In these trials, no differences in residual H_2O_2 concentration between treated apples and controls were found, either in the cut fruit or in juice made therefrom.

Cantaloupe Decontamination. Decontamination trials were carried out with composite cantaloupe rind plug samples to compensate for the extensive variability seen in bacterial loads on external surfaces of individual melons. Washes with 1000 ppm Cl_2 and various commercial surfactant/sanitizer solutions were generally ineffective in reducing bacterial populations (Table

3, Expt. A). However, combinations of 5% H_2O_2 with Sanitizers B, D and E achieved a 2-3 log reduction (Expt. B).

When washing treatments were applied to uncut cantaloupe prior to preparation of fresh-cut melon cubes, bacterial growth and spoilage during storage could be suppressed by a two-stage treatment (Table 4). In Expt. C, an acidulant/surfactant wash, followed by treatment with 5% H_2O_2 at 60°C, was effective in suppressing bacterial growth and spoilage for at least 14 days at 4°C. In Expt. D, an acidulant/surfactant/H_2O_2 wash, followed by treatment with 2000 ppm Cl_2, suppressed bacterial growth, but an improvement in shelf-life over that obtained with Cl_2 alone could not be demonstrated. Further research is needed to optimize these treatments and determine their effects on human pathogens such as *Salmonella*.

Treatment of sprouts with hydrogen peroxide. Bacterial populations (aerobic plate counts) on seeds generally were between 10^3 and 10^4 cfu/g; however, counts on freshly produced sprouts were as high as 10^9 cfu/g (data not shown). Application of 200 ppm H_2O_2 to sprout samples in 3 successive 1 min treatments (simulating watering during sprout production) reduced the bacterial load by less than 1 log (Table 5). Catalase-catalyzed breakdown of H_2O_2 following exposure accounted for the observed gas (O_2) evolution, and only trace H_2O_2 residues remained after 1 h. Ascorbic acid retention was between 60 and 80%, losses resulting from oxidation by H_2O_2 and leaching. Sprouts given a single application of 5% H_2O_2 for 30 sec or 2 min showed about a 1 log reduction in the aerobic plate count, irrespective of exposure time (Table 6). However, peroxide residues remaining after 10 min were larger with the longer exposure time. Ascorbic acid retention varied between 80 and 100%. Application of 5% H_2O_2 to seed samples for 1 min had little effect on the aerobic plate count (data not shown).

Because of the large amplification in bacterial populations that occurs during seed germination and sprout growth and the ineffectiveness of H_2O_2 in decontaminating seeds, treatments to control bacterial populations should be applied during or after sprout production. Our results indicate that application of 5% H_2O_2 after sprout production will reduce the bacterial load by only about 90%. However, continuous application of more dilute H_2O_2 during sprout production might be more successful in suppressing bacterial growth without adversely affecting sprout quality. This approach is under investigation.

CONCLUSIONS

Combinations of H_2O_2 with commercial washing formulations show promise in decontaminating apples containing *E. coli* and in reducing the microbial load on cantaloupe melons. However, alternative decontamination methods are needed for seeds and sprouts.

REFERENCES

Anon. 1996. Bibliography. National Advisory Committee on Microbiological Criteria for Foods. Fresh Produce Subcommittee.

Anon. 1997. Outbreaks of *Escherichia coli* O157:H7 infection associated with eating alfalfa sprouts — Michigan and Virginia, June-July 1997. MMWR 46: 741-744.

Ayhan, Z., Richter, E. R., Yousef, A. E., and Chism, G. W. 1996. Preservation of fresh cut melons. Presented at 1996 Annual Meeting of Institute of Food Technologists, New Orleans.

Beuchat, L. R. 1996. Pathogenic microorganisms associated with fresh produce. J. Food Protection 59: 204-216.

Brackett. R. E. 1992. Shelf stability and safety of fresh produce as influenced by sanitation and disinfection. J. Food Protection 55: 808-814.

Cantwell, M., López-Gálvez, G., Rovelo-González, J., and Nie, X. 1996. Factors affecting quality of minimally processed cantaloupe melon. I. Temperature and atmosphere. Presented at 1996 Annual Meeting of Institute of Food Technologists, New Orleans.

Garg, N., Churey, J. J., and Splittstoesser, D. F. 1990. Effect of processing conditions on the microflora of fresh-cut vegetables. J. Food Protection 53: 701-703.

Mahon, B. E., Pönkä, A., Hall, W. N., Komatsu, K., Dietrich, S. E., Siitonen, A., Cage, G., Hayes, P. S., Lambert-Fair, M. A., Bean, N. H., Griffin, P. M. 1997. An international outbreak of *Salmonella* infections caused by alfalfa sprouts. J. Infectious Diseases 175: 876-882.

O'Mahony, M., Cowden, J., Smyth, B., Lynch, D., Hall, M., Rowe, B., Teare, E. L., Tettmar, R. E., Rampling, A. M., Coles, M., Gilbert, R. J., Kingcott, E., and Bartlett, C. L. R. 1990. An outbreak of *Salmonella saint-paul* infection associated with beansprouts. Epidemiol. Infect. 104: 229-235.

Pönkä, A., Anderson, Y., Siitonen, A., de Jong, B., Jahkola, M., Haikkala, O., Kuhmonen, A., and Pakkala, P. 1995. *Salmonella* in alfalfa sprouts. Lancet 345: 462-463.

Prokopowich, D. and Blank, G. 1991. Microbiological evaluation of vegetable sprouts and seeds. J. Food Protection 54: 560-562.

Sapers, G. M., Miller, R. L., Miller, F. C., Cooke, P. H., and Choi, S.-W. 1994. Enzymatic browning control in minimally processed mushrooms. J. Food Sci. 59: 1042-1047.

Sapers, G. M., Miller, R. L., and Simmons, G. 1995. Effects of hydrogen peroxide treatment on fresh-cut fruits and vegetables. Presented at 1995 IFT Annual Meeting, June 3-7, Anaheim, CA (Abstract).

Simmons, G. F., Rij, R., Smilanick, J. L., and John, S. 1996. Microbial population reduction on cantaloupe destined for the minimally processed market using hydrogen peroxide or SO_2. Presented at 1996 Annual Meeting of American Society for Horticultural Science, Lexington, Kentucky, October 6-10.

Wei, C. I., Huang, T. S., Kim, J. M., Tamplin, M. L., and Bartz, J. A. 1995. Growth and survival of *Salmonella montevideo* on tomatoes and disinfection with chlorinated water. J. Food Protection 58: 829-836.

Zhuang, R.-Y., Beuchat, L. R., and Angulo, F. J. 1995. Fate of *Salmonella montevideo* on and in raw tomatoes as affected by temperature and treatment with chlorine. Appl. Environ. Microbiol. 61: 2127-2131.

Table 1—Effect of commercial sanitizing agents on *E. coli* (ATCC 25922) in inoculated Golden Delicious apple halves[a]

Treatment	Composition	pH	n	Log_{10} Reduction
200 ppm Cl_2 (pH 6.5)	--	6.5	5	2.07 ± 0.31
1% Sanitizer A	Acid anionic surfactant	2.4	2	1.93 ± 0.02
1% Sanitizer A at 50°C	Acid anionic surfactant	2.4	2	2.14 ± 0.04
5% Sanitizer B at 50°C	Acid soap	3.4	2	1.99 ± 0.35
1% Sanitizer C	Phosphoric acid + surfactant	2.1	2	2.04 ± 0.00
1% Sanitizer C at 50°C	Phosphoric acid + surfactant	2.1	2	2.30 ± 0.26
1% Sanitizer D	Phosphoric acid + surfactant	1.7	2	1.90 ± 0.11
1% Sanitizer D at 50°C	Phosphoric acid + surfactant	1.7	2	2.61 ± 0.11
1.6% Sanitizer E	Phosphoric acid + surfactant	1.9	2	1.97 ± 0.56
3.2% Sanitizer E	Citric acid + surfactant	2.3	2	2.10 ± 0.32
4% Sanitizer G	Trisodium phosphate	12.4	3	2.36 ± 0.08
4% Sanitizer G at 50°C	Trisodium phosphate	12.4	2	2.45 ± 0.08
1% Sanitizer H	Surfactant	9.3	2	0.98 ± 0.07
1000 ppm Sanitizer I	Peracetic acid + H_2O_2 + acetic acid	3.3	2	2.05 ± 0.48
1000 ppm Sanitizer I at 50°C	Peracetic acid + H_2O_2 + acetic acid	3.3	2	2.58 ± 0.22

[a]For each treatment, 9 apples cut in half, inoculated by immersion for 5 min in 3L diluted *E. coli* inoculum containing approx. 2.0×10^7 cfu/mL, and washed for 1 min.

Table 2— Efficacy of washes containing hydrogen peroxide and commercial sanitizing agents on decontamination of Golden Delicious apple halves inoculated with *E. coli* (ATCC 25922)[a]

Treatment	n	Log_{10} Reduction
200 ppm Cl_2 (pH 6.5)	5	2.01 ± 0.17
2.5% H_2O_2	2	2.74 ± 0.43
5% H_2O_2	4	3.39 ± 0.39
5% H_2O_2 at 50°C	3	3.82 ± 0.82
5% H_2O_2 + 5% Sanitizer B at 50°C	2	>4.08
5% H_2O_2 + 1% Sanitizer D	2	3.27 ± 0.21
5% H_2O_2 + 1% Sanitizer D at 50°C	2	4.20 ± 0.56
5% H_2O_2 + 2% Sanitizer D at 50°C	2	>4.08
5% H_2O_2 + 1.6% Sanitizer E at 50°C	3	3.82 ± 0.65
5% H_2O_2 + 3.2% Sanitizer F at 50°C	2	3.63 ± 0.28
5% H_2O_2 + 2% Sanitizer G	2	3.27 ± 0.29
5% H_2O_2 + 2% Sanitizer G at 50°C	2	3.55 ± 1.67[b]
5% H_2O_2 + 1% Sanitizer H	2	3.22 ± 0.20
5% H_2O_2 + 1% Sanitizer H at 50°C	2	3.20 ± 0.16

[a]For each treatment, 9 apples cut in half, inoculated by immersion for 5 min in 3L diluted *E. coli* inoculum containing approx. 1.3×10^7 cfu/mL, and washed for 1 min.
[b]Variable response due to decomposition of heated alkaline H_2O_2.

Table 3—Effect of sanitizing agents on bacterial population of cantaloupe plugs.

Expt.	Treatment[a]	Exposure Time (min)	Mean Log_{10} Reduction[b]
A	1000 ppm Cl_2 (pH 6.5) at 50°C	5 min	0
	4% Sanitizer G at 50°C	5 min	1.55 ± 0.18
	2% Sanitizer I at 50°C	5 min	1.14 ± 0.13
B	5% H_2O_2 + 2% Sanitizer B at 60°C	1.5 min	1.94 ± 0.40
	5% H_2O_2 + 2% Sanitizer D at 60°C	1.5 min	2.96 ± 0.55
	5% H_2O_2 + 2% Sanitizer E at 60°C	1.5 min	2.33 ± 0.00

[a]10 cantaloupe rind plugs stirred in 500 mL solution, then rinsed.
[b]Mean ± Stand. Dev. for duplicate determinations; based on reduction in aerobic plate count (cfu/cm^2).

Table 4—Effect of sanitizing treatments, applied to uncut cantaloupes, on microbiological quality of fresh-cut melon cubes stored at 4°C.

Expt.	Treatment	Log_{10} cfu/g Day 0	Day 7	Day 14	Onset of Visual Spoilage (Day)[b]
C	Control	4.06	6.45	9.93	7
	1000 ppm Cl_2 (pH 6.5)	3.88	7.72	9.86	11
	2% Sanitizer D + 5% H_2O_2 at 60 C	4.26	6.31	9.72	11
	2% Sanitizer D at 60°C; 5% H_2O_2 at 60°C[a]	3.89	4.78	4.70	18
D	Control	3.64	6.18	8.64	14
	2000 ppm Cl_2 (pH 6.5)	2.32	4.72	7.84	17
	2% Sanitizer E + 5% H_2O_2 at 60°C	3.84	4.34	7.63	17
	2000 ppm Cl_2 (pH 6.5); 2% Sanitizer E + 5% H_2O_2 at 60°C[a]	3.41	4.60	8.02	17
	2% Sanitizer E + 5% H_2O_2 at 60°C; 2000 ppm Cl_2 (pH 6.5)[a]	3.11	3.60	5.85	17

[a]Two-stage treatment.
[b]Slime formation, visible colonies or off-odor.

Table 5—Log reduction, gas evolution, residual peroxide and ascorbic acid retention in sprouts given 3 successive 1 min treatments with 200 ppm H_2O_2.

Sample	Log Reduction Aerobic Plate Count	Gas Evolution	Residual H_2O_2 (ppm) 1 min	1 hr	Ascorbic Acid Retention (%)
Alfalfa-1	0.34	Slight	0	0	69
Alfalfa-2	0.22	Slight	2	0	61
Radish	0.21	Slight	2.5	0	84
Garlic/alfalfa	0.32	Slight	2	0	70
Sunflower	0.25	None	2	<2	73
Lentil	0.26	Slight	0	0	65

Table 6—Log reduction, gas evolution, residual peroxide and ascorbic acid retention in sprouts treated with 5% H_2O_2.

Sample	H_2O_2 Exposure (sec)	Log Reduction Aerobic Plate Count	Gas Evolution	Residual H_2O_2 at 10 min (ppm)	Ascorbic Acid Retention (%)
Alfalfa	30	0.87	Vigorous	<0.5	82
	120	1.03	Vigorous, foam	2	77
Garlic/alfalfa	30	1.07	Vigorous	0.5-2	96
	120	1.07	Vigorous, foam	2	104